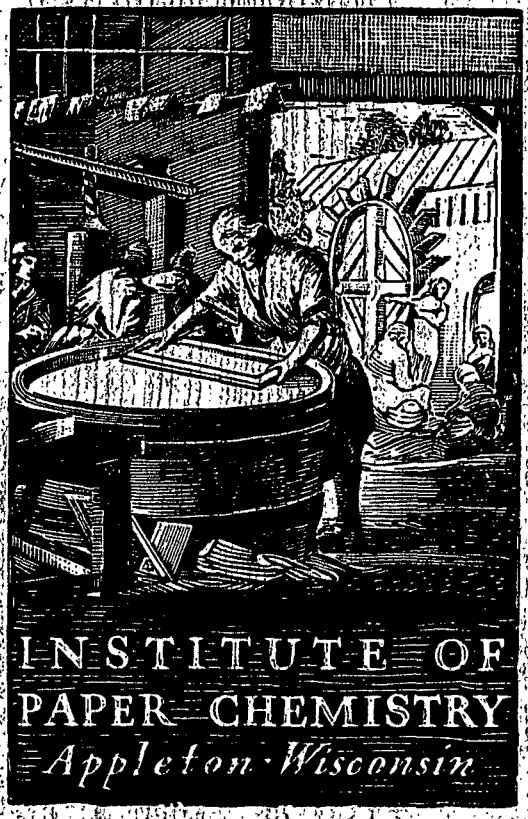

PRODUCTION AND INTENSIVE MANAGEMENT OF GENETICALLY IMPROVED ASPEN

Project 3537

Report Three
A Progress Report
to
MEMBERS OF GROUP PROJECT 3537

February 25, 1988

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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Blandin Paper Co.

Consolidated Papers, Inc.

Michigan Department of Natural Resources

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THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

PRODUCTION AND INTENSIVE MANAGEMENT OF GENETICALLY IMPROVED ASPEN

SUMMARY

Seventeen hybrid crosses were made for seedling production by cooperators and IPC. Eight small crosses were made for hypoxylon screening; seven hybrid backcrosses were made following discussions with other aspen tree improvement workers who described the exceptional growth rate and form achieved from backcrossing. Two crosses were also made for the IPC tissue culture team to test the possibility of using pollen as a vector to introduce a foreign gene.

325,000 Triploid hybrid seeds were produced. Ta-10, the tetraploid male used in producing triploids, flowered heavily and a quantity of pollen was stored for use in 1988. Female flowers were collected from the Packaging Corporation seed orchard.

IPC produced 10,500 triploid hybrid seedlings along with several hundred seedlings from hybrid backcrosses and several hundred ramets from clonal selections. Work to improve the seedling to seed ratio by encapsulation of seed is planned. Seedling storage was also examined this past year and recommendations for fall lifting, packing, and freezer storage were developed.

Site selection and establishment recommendations for hybrid aspen were summarized. Soil texture and nutrient requirements were described along with planting methods and stock handling recommendations.

Four replicated trials were measured: a triploid hybrid sucker stand resulting from second cutting of 23-year-old suckers; a new trial evaluating both hybrid clones and seedlings; a trial evaluating effects of fertilization

and irrigation on a natural sucker stand initiated in 1970 which will be discussed in next year's annual report; a planting of 38 hybrid poplar clones was measured for growth and disease information and the results reported at the 5th North Central Tree Improvement Conference.

Three demonstration plantings of hybrid aspen were planted, two with Consolidated Papers and one with the Michigan DNR. Growth and survival were good in the Consolidated plantings, but damage by a logger occurred in the Michigan DNR planting. The Consolidated plantings were operational trials, hand-planted by a professional crew.

Twenty-two clonal selections were made this year, of which all but two were over 20 years of age. Parentage ranged from tremuloides triploid hybrids, to bigtooth hybrids. An exceptional natural clone selected in 1966 was brought to our attention by a logger who noted our tree tag and called before cutting. It will be propagated and included in future plantings.

Fifteen hybrid selections made in 1986 were propagated for field testing. The number of root sprouts produced from several clones was determined by recording the length and diameters of roots collected and counting the number of sprouts. Tissue culture propagation of 13 of these clones was also initiated. Variation in shoot production between clones in culture was noted. The performance of slower shoot producing clones could be modified by changing culture conditions.

Hypoxylon bioassay work continued at a slower pace. Efforts were concentrated on analyzing extant data and checking correlations between variables such as growth rate, periodic incidence of hypoxylon, and hypoxylon associated

mortality. A new bioassay technique using fungus mycelium to infect aspen seedlings will be examined. Present plans call for collecting and isolating several new Hypoxylon mammatum inoculum sources.

INTRODUCTION

Interest in the use of hybrid aspen to remedy an age class imbalance in native aspen is being expressed by cooperators, former cooperators, and the State of Minnesota. However, to have an impact, methods to increase seed production and seed efficiency must be put in place.

The importance of the aspen resource is underscored by the fact that approximately half of the roundwood cut in the Lake States is aspen. The other half is made up of the remaining hardwood and softwood species. Utilization trends indicate even greater use of aspen, particularly for waferboard and oriented strand board production.

Our success in establishing plantations indicates that hybrid aspen seedlings can be handled in a manner not greatly different than that for other species. Stock handling methods, site selection guidelines, establishment procedures, and proven genetic material are available. Improved seed production and seedbed techniques will make available more seedlings for operational plantings.

1987 CROSSING PROGRAM

The 1987 breeding work emphasized the production of hybrid seed for lanting stock. Small quantities of P. tremuloides seed were also produced or hypoxylon screening tests. Thirty-four crosses were made: nine diploid and ight triploid hybrid for planting stock; eight for hypoxylon screening; seven ybrid backcrosses; and two crosses for our Cell and Tissue Culture Group. able 1 summarizes the crosses, and Table 2 summarizes 1987 seed production.

Hybrid backcrosses were made after foreign cooperators commented on the ncreased hybrid vigor that occurred when selected clones were backcrossed to ne of the parental species or crossed with another hybrid. A number of diploid ybrids selected for clonal trials were used to produce the seven hybrid ackcrosses, five of which were diploid hybrid P. tremuloides x P. tremula emales crossed with diploid P. tremuloides males.

The tissue culture group was interested in using pollen as a vector to ntroduce a foreign gene into a plant. The gene for kanamycin (an antibiotic) esistance was available. Because of the relative ease in which controlled rosses could be made and the short period of time reqiured to produce seed, spen was selected as the test organism. A second cross was also made as a ontrol. Results are being evaluated to determine if the gene was incorporated. roject 3537 funds were not used for this work.

YPOXYLON SCREENING CROSSES

The eight crosses made for hypoxylon bioassay screening have been tored for future work. Crosses involving specific parentage combinations are ade when flowers are available. It is hoped that these and other stored rosses can be used with Hypoxylon mammatum isolates selected from infected

Table 1. Summary of 1987 crosses and location of parent trees.

Cross Number[a]	Parents (Female x Male)		
XT-1-87	T-1-59 (Clintonville, WI)	x	T-46-60 (Ralph, MI)
XT-Ta-2-87	T-53-60 (Fern, WI)	x	Ta-10 (Ekebo, Sweden)
XT-3-87	T-1-58 (Ontonagon, MI)	x	Clone 7 (Wausau, WI)
XT-4-87	T-1-58 (Ontonagon, MI)	x	T-6-67 (Porcupine Mtn., MI)
XT-5-87	T-1-58 (Ontonagon, MI)	x	XT-22-56-S-3 (IPC Nursery)
XT-6-87	XT-22-56-S-2 (IPC Nursery)	x	XT-32-56-S-4 (IPC Nursery)
XT-7-87	T-1-58 (Ontonagon, MI)	x	T-44-60 (Ralph, MI)
XT-8-87	T-1-58 (Ontonagon, MI)	x	T-6-61 (Fern, WI)
XT-Ta-9-87	T-1-58 (Ontonagon, MI)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-10-87	T-50-60 (Ralph, MI)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-11-87	Clone 5 (Wausau, WI)	x	Ta-10 (Ekebo, Sweden)
XTTa-T-12-87	XT-Ta-65-60, S-2 (Clintonville, WI)	x	T-44-60 (Ralph, MI)
XTTa-T-13-87	XT-Ta-65-60, S-2 (Clintonville, WI)	x	Ta-6-61 (Fern, WI)
XTTa-T-14-87	XT-Ta-61-60, S-2 (Clintonville, WI)	x	T-44-60 (Ralph, MI)
XTTa-T-15-87	XT-TA-61-60, S-2 (Clintonville, WI)	x	T-6-61 (Fern, WI)
XTa-T-16-87	Ta-8-68 (Germany)	x	T-44-60 (Ralph, MI)

Table 1 (Contd.) Summary of 1987 crosses and location of parent trees.

Cross Number[a]	Parents (Female x Male)		
XTDa-T-17-87	XT-Da-18-59, S-2 (Sugar Camp, WI)	x	T-44-60 (Ralph, MI)
XTDa-T-18-87	XT-Da-18-59, S-2 (Sugar Camp, WI)	x	T-6-61 (Fern, WI)
XT-Ta-19-87	T-11-68 (Eagle River, WI)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-20-87	T-1-59 (Clintonville, WI)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-21-87	T-2-87 (IPC Nursery)	x	Ta-10 (Ekebo, Sweden)
XT-Ta-22-87	T-1-87 (IPC Nursery))	x	Ta-10 (Ekebo, Sweden)
XT-Ta-23-87	T-80-57 (Alston, MI)	x	Ta-1-87 (Germany)
XT-Ta-24-87	T-80-57 (Alston, MI)	x	Ta-2-87 (Germany)
XT-25-87	T-80-57 (Alston, MI)	x	T-46-60 (Ralph, MI)
XT-Ta-26-87	T-80-57 (Alston, MI)	x	Ta-1-75 (East Prussia)
XT-Ta-27-87	Clone 2 (Wausau, WI)	x	Ta-1-87 (Germany)
XT-Ta-28-87	Clone 2 (Wausau, WI)	x	Ta-2-87 (Germany)
XT-TTa-29-87	Clone 2 (Wausau, WI)	x	XT-Ta-14-58, S-28 (Sugar Camp, WI)
XT-Ta-30-87	T-3-78 (IPC Nursery)	x	Ta-1-75 (East Prussia)
XT-31-87	T-3-78 (IPC Nursery)	x	T-44-60 (Ralph, MI)
XT-32-87	T-3-78 (IPC Nursery)	x	T-46-60 (Ralph, MI)
XT-Ta-33-87	T-20-56 (Watersmeet, MI)	x	Ta-2-87 (Germany)
XT-Ta-34-87	T-12-58 (Clintonville, WI)	x	Ta-2-87 (Germany)

[a]See Appendix for description of crossing code.

Table 2. Summary of 1987 seed production.

Cross[a]	Number of Catkins Pollinated	Number of Catkins Collected	Number of Seeds	Seeds/ Catkins Pollinated	Germination, %	Purpose
XT-1-87	15	15	1,715	114	--	Hypoxylon screening
XT-Ta-2-87	17	16	1,070	67	--	3n seed production
XT-3-87	19	19	1,590	84	--	Hypoxylon screening
XT-4-87	17	17	3,138	185	--	Hypoxylon screening
XT-5-87	15	14	1,439	103	--	Hypoxylon screening
XT-6-87	13	6	3,824	637	--	Hypoxylon screening
XT-7-87	--	--	--	--	--	Pollen transformation of aspen
XT-8-87	18	16	460	29		Control for XT-7-87
XT-Ta-9-87	742	656	77,845	119	92	3n seed production
XT-Ta-10-87	397	385	118,919	309	86	3n seed production
XT-Ta-11-87	44	42	11,008	262	96	3n seed production
XTTa-T-12-87	110	104	97,574	938	95	Testing
XTTa-T-13-87	114	103	73,031	709	91	Testing
XTTa-T-14-87	96	93	67,817	729	97	Testing
XTTa-T-15-87	97	93	34,270	368	82	Testing
XTa-T-16-87	24	14	10,278	734	--	Testing
XTDa-T-17-87	47	40	14,643	366	98	Testing
XTDa-T-18-87	46	39	19,206	492	--	Testing
XT-Ta-19-87	284	115	18,867	164	72	3n seed production
XT-Ta-20-87	452	380	32,125	85	59	3n seed production
XT-Ta-21-87	276	74	4,180	56	--	Testing
XT-Ta-22-87	308	258	63,970	248	78	3n seed production
XT-Ta-23-87	52	50	0	0	--	Testing
XT-Ta-24-87	48	46	0	0	--	Testing
XT-25-87	24	24	25	1	--	Testing
XT-Ta-26-87	48	46	0	0	--	Testing
XT-Ta-27-87	156	117	25	< 1	--	Testing
XT-Ta-28-87	116	118	25	< 1	--	Testing
XT-TTa-29-87	55	42	25		--	Testing
XT-Ta-30-87	59	55	6,010	109	92	Testing
XT-31-87	32	26	1,192	82	--	Testing
XT-Ta-33-87	542	278	8,241	30	82	Experiment with boxed aspen grafts for producing seeds
XT-Ta-34-87	161	54	843	16	--	

trees in full-sib families and from natural stands. Information on the current work with the hypoxylon bioassay can be found in the Hypoxylon Research section of this report.

TRIPLOID HYBRID SEED PRODUCTION

The 1987 crossing program again emphasized the production of triploid hybrid seed for planting stock. Approximately 325,000 triploid seeds were produced. The availability of pollen from the tetraploid male clone Ta-10 was good and a quantity was stored for use in this year's crossing program. The female flowers were obtained from the former Packaging Corporation seed orchard near Freesoil, Michigan and from the Institute's Greenville arboretum.

The orchard at Freesoil began producing significant amounts of female flowers at age eight and at least one of the four female clones has flowered each year since then. The male tetraploid clone within that orchard was severely damaged by rodents during the establishment phase and was not replaced by PCA before the land was sold. Male flowers have been observed on the scattered, surviving ramets, and pollen was being shed during the receptive period of the female clones in the orchard. Unfortunately, too few male ramets have survived to produce hybrid seed.

The Freesoil orchard has demonstrated early seed production possibilities from well-established clones. Had the male clone been successfully established, useable quantities of seed would most likely be available. Clearly, the care and attention given to establishment of hybrid aspen orchards will lead to significant, early flower production.

1987 SEEDBEDS

Hybrid aspen seed was sown in the IPC nursery at Greenville, in two small seedbeds at Blandin Paper Co. nursery in Grand Rapids, Minnesota, and in a small seedbed in the Michigan DNR nursery in Manistique, Michigan. Good seedling development, although somewhat low bed densities, occurred at the Blandin nursery and a hail storm damaged some seedlings in late summer. Insufficient germination occurred at the MDNR nursery, apparently caused by herbicide treatment (Goal) two weeks prior to sowing. Good germination and seedbed development occurred at the IPC nursery at Greenville (Fig. 1). Blandin also had 35,000 seedlings contract grown in containers.

IPC SEEDBEDS

Table 3 summarizes the 1987 seedbed and clonal production of hybrid aspen. Approximately 10,500 triploid hybrids were grown along with several hundred ramets from hybrid clones and seedlings from hybrid backcrosses (Fig. 1). The crosses with small numbers of seedlings were grown for testing in replicated trials and represent new types of hybrids. The clonal stock (those with "S" numbers) represents initial increase of clones from root suckers of outstanding field selections. These ramets will be tested in clonal trials and will provide roots for further cloning next year.

The number of hybrid aspen seedlings produced from a given number of seeds was evaluated again this year. The production of five plantable seedlings per square foot of nursery bed has been the target density for all seedbeds. To reach that number, 25 seeds per square foot are sown. Generally, the target number of plantable seedlings is not reached, and conversion ratios typically are 25 percent or less.

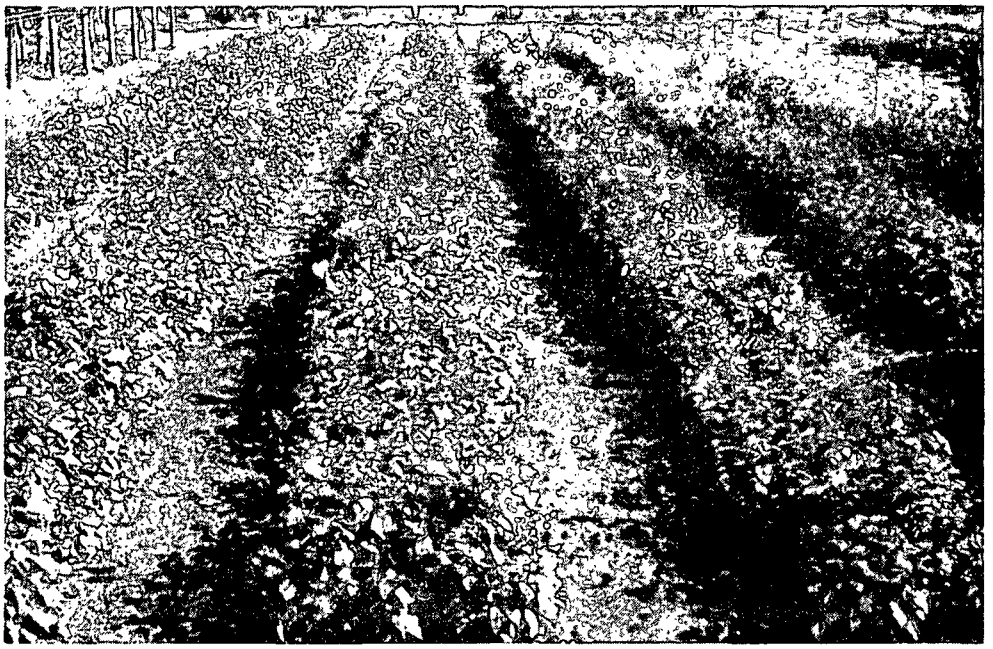

Figure 1. The 1987 hybrid aspen seedbeds produced 10,500 plantable seedlings and several hundred ramets from clonal selections. The beds produced 4 plantable seedlings/ft^2.

Aspen seedlings are very sensitive to seedbed conditions during the irst weeks following sowing; any drying of the surface results in drastically owered seedling numbers. Even under the best of conditions the return of plan- able seedlings per seed sown is low. Any improvement that increases the sur- ival of that extremely small seedling will increase the number of seedlings per nit sown seed. The recent ability to encapsulate small seeds such as celery nd tobacco (both of which are smaller than aspen seed) and encapsulation of issue culture produced embryos would seem to have application with aspen seed. ncapsulation may increase survival of germinating seedlings in seedbeds by oviding a matrix to hold moisture and reduce the effects of seedbed drying. ne of the drawbacks in using aspen in container operations is the difficulty in owing one seed per container.

Table 3. 1987 IPC seedling and clonal production.

Material	Total No. Plantable	No. Undersized
XT-Ta-9-87	2,507	987
XT-Ta-10-87	5,209	2,200
XT-Ta-20-87	2,711	900
XT-17-84	350	135
XT-4-86	1,588	500
XT-Ta-13-86	144	60
XTa-T-14-86	365	150
XTa-T-23-86	428	200
XT-Ta-11-87	301	164
XTTa-T-12-87	288	148
XTDa-T-17-87	383	200
XT-31-87	657	264
Raverdeau	650	--
XT-Ta-10-58-S-5	467	--
XT-Ta-14-58-S-3	123	19
XT-Ta-14-58-S-23	133	39
S-24	48	6
S-25	10	24
S-26	35	10
S-27	46	15
S-28	10	12
S-29	41	16
XT-Ta-6-64-S-1	280	80
XT-Ta-10-69-S-1	470	100
XT-Ta-10-69-S-2	183	70
XCA-T-1-86-S-1	85	5
XCA-G-38-67-S-1	10	9
XCA-G-38-67-S-2	110	36
XT-33-68 #201 (201-68)	34	8
XT-217-72 #22 (22-72)	23	37

By increasing the size of the seed through the use of an encapsulating aterial, it will be easier to sow containers mechanically. Encapsulation hould result in a more efficient use of current seed production. Commercial as ell as experimental encapsulators are being contacted, and we intend to test ncapsulation this year.

In addition to improving seed use efficiency, seedling handling has een examined. For the past two years, seedlings have been fall lifted after eaf drop, graded, counted, and placed in waxed corrugated containers with damp phagnum moss. The boxes of seedlings are then taken to a commercial cold torage facility and maintained at 30°F until needed in the spring. This type of torage was used to a limited extent for a number of years before the removal of he barn that served as the main storage facility. Field performance of stock andled in this manner has been very good with no adverse effects noted. The lexibility gained in handling stock in the spring to reduce the effect on stock uality from planting delays, and the one time handling of stock in the fall uring lifting are definite advantages.

HYBRID ASPEN SITE SELECTION AND PLANTATION ESTABLISHMENT TECHNIQUES

Questions frequently arise regarding aspen establishment. A brief description of techniques, drawn from experiences of both cooperators and IPC, was put together as a guideline.

SITE SELECTION

A number of early plantations failed because of the site selected for planting. A combination of low soil fertility and moisture often led to mortality. It must be recognized that there is a lower limit to site quality from which successful establishment can be expected. Experience indicates those sites best suited for aspen are low to medium quality, mesic, hardwood sites with soil textures from loamy sands to silt loams. Sandy soils and loamy sands with low levels of silt and clay are not acceptable, nor will aspen or aspen hybrids do well on poorly drained soils. Soil nutrient guidelines, given in previous reports, are presented again in Fig. 2. Note that the guidelines do not consider moisture availability.

Early in the aspen program, small plantings were frequently placed on old fields. Those that were successful received considerable mechanical cultivation - an uneconomical procedure. Most of those old field plantings failed through a combination of factors: heavy grass competition for moisture; rodent, deer, and/or rabbit damage; white grub injury to root systems; and inability to recover from Saperda calcarata (long-horned beetles) stem galls.

Plantings in hardwood clearcuts had few such problems and were most successful. Planting must be done as soon after harvesting as possible to avoid competition with returning vegetation.

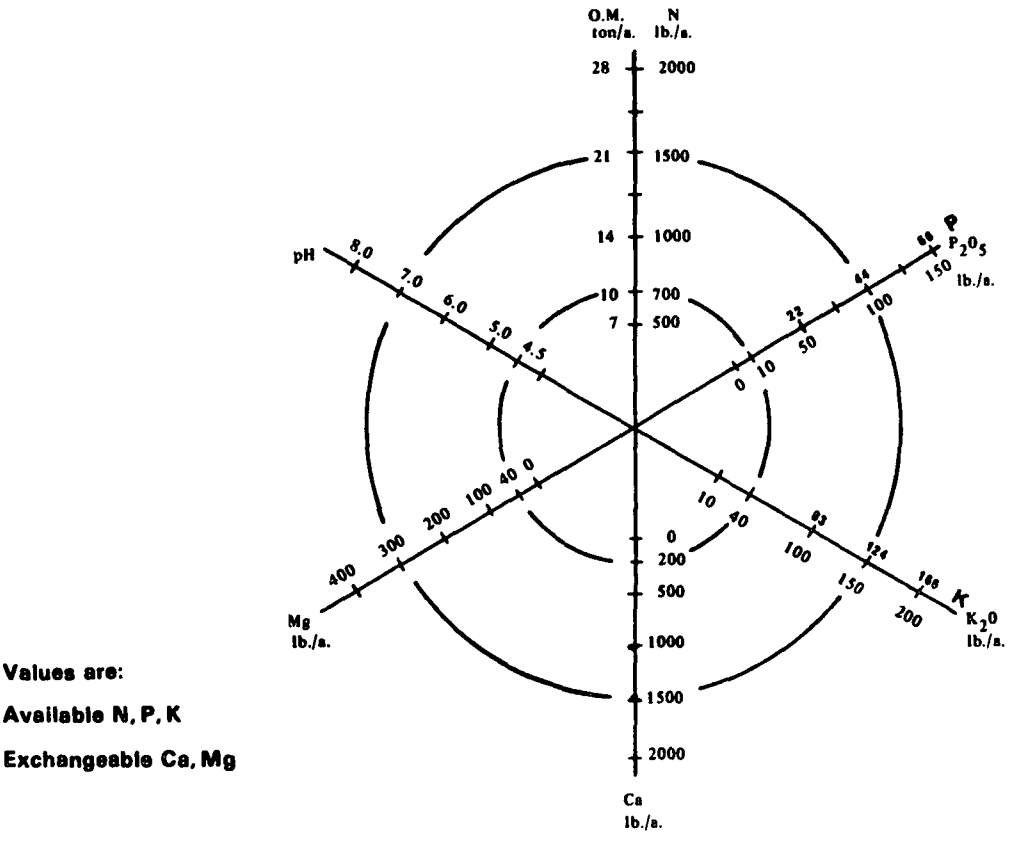

Figure 2. A soil nutrient guideline was developed over the course of the aspen project and reflects soil analyses taken near selected trees and from widespread plantations and natural stands of aspen. The inner circle denotes the minimum level of soil nutrients required for growth, and the outer circle denotes nutrient levels beyond which increased growth could not be expected. The guideline does not take into account moisture availability.

Landings, natural openings, problem areas where nothing else has grown, and old fields with no site preparation are to be avoided. Hybrid aspen is not the answer for problem sites.

Situations where planted aspen is the most readily available food source such as the only hardwood near conifers, small old field plantings where deer travel up and down rows feeding repeatedly, and small plantings near

standing timber are to be avoided. If only small quantities of trees are available for planting, place them in the center of the planting site rather than on the edge.

ESTABLISHMENT METHODS

Best success will result when a fall or winter hardwood clearcut is followed by spring planting. Good quality stock properly planted will outgrow returning hardwood competition and herbaceous competition. Site preparation in such cases requires little more than moving slash to facilitate planting; whole tree chipping, such as that used in fuel operations, is ideal. Disk trenching on these kinds of sites has also been successful.

There are no chemical treatments recommended for release from hardwood competition, thus the need to plant before such competition has a chance to develop.

Furrowing or deep disk trenching can present problems with mice and voles, and seedlings are best planted on the side rather than in the bottom of the trench.

Chemical treatments include the use of Oust at 1-2 ounce rates banded during disk trenching. There are some indications that Oust can be sprayed over the top of aspen for release when applied as a preemergent at 1-2 ounce rates, but caution is urged. Hybrid aspen has also been planted successfully in the season after chemical site preparation treatments with Velpar, Oust, and Arsenal. Planting in Round-up treated areas as soon as one week after treatment is also possible. Fusilade has also been successfully sprayed over aspen for grass control.

STOCK QUALITY

Although stock quality and handling are areas of importance, they are frequent areas of neglect. Hardwood planting stock root systems do not have the fibrous configuration typical of conifers. As such they are more difficult to plant, particularly if they have not been properly root pruned at the nursery. Both tops and roots should be pruned before leaving the nursery. As with any seedling, the roots should not be allowed to dry, nor should they be exposed to heat. The stock must be dormant when planted. Hybrid aspen stock exposed to heat will rapidly break dormancy. Survival and vigor decline rapidly if buds have flushed.

PLANTING METHODS

Properly pruned stock can be machine or hand planted with little modification of existing equipment. Poor planting success is often due to excessive root systems. Pruned stock was efficiently hand planted with "hodads" by a professional contract planting crew this past spring.

As with other types of stock, aspen should not be twisted into holes or "J" rooted (a problem frequently encountered with unpruned stock). Aspen stock should be deep-planted whenever possible with the root collar 1-3 inches below ground level. This increases the likliehood that soil moisture will be available to developing root systems, and promotes rooting above the root collar as well. Stock should be planted as near to vertical as possible but no more than 15 degrees from vertical to avoid excessive sweep and potential windthrow problems. Recommended spacing remains the 9 x 9 feet used in almost all of the plantings and trials put in over the course of the project. Plant bareroot

stock as early in the spring as possible. There is some indication that container stock may be suitable for fall planting in areas with sufficient snow cover to reduce frost heaving.

FIELD TRIAL EVALUATION

Four replicated field trials were measured this fall. One evaluates ıe suckering ability of aspen and aspen hybrids; one is a newly planted trial valuating both clones and hybrid seedlings; and one completes evaluation of ertilization and irrigation treatments on a natural aspen sucker stand. This atter trial (STV) will be included in next year's annual report. A computer rogram to handle the volume data was not completed in time for this year's eport. The fourth was a hybrid poplar clonal trial measured for a paper pre- ented at the 5th North Central Tree Improvement Conference. As discussed ast year, space is needed to test a number of very promising hybrids and clones)eing selected for potential plantation use.

EXPERIMENTAL TRIAL X

Trial X is located on the Ripco test area in Oneida County, Wisconsin. It compared the growth and suckering of two triploid hybrid crosses, two native triploid clones, and two diploid crosses. The trial had a four replicate, randomized-block design with three replications cut at age five to form a suckering study; the fourth replication was left intact.

The trial was cut in the fall of 1986 for the second time. The sucker stand portion of the trial was 23 years of age and the intact replication was 28 years. Several superior individuals were selected prior to harvesting and have been propagated.

A series of 24, 1/1000 acre plots were put in on the trial, one at the center of each block of test trees. The numbers of stems and heights were recorded. Table 4 summarizes the growth and number of stems one year after

harvesting. It should be noted that hybrid suckering occurred throughout the trial following the first cutting, and has outgrown and replaced most of the suckering from the original materials in the trial. Returning suckers within the previously uncut replication are primarily hybrid. An evaluation of the proportion of hybrid suckers on the area will be undertaken this coming year. The size and number of returning hybrid suckers demonstrates the ability of hybrid aspen, once established, to convert an area.

Table 4. Average height and number of stems per acre one year after harvesting Trial X.

Original Material in Which Plots Were Located	Av. Number Stems/Acre	Av. Number Stems/Acre > 3 Ft.Ht.	Av. Ht. Ft. All Stems	Av. Ht. Stems > 3 Ft.
T-66A-57	38,000	15,000	2.8	4.2
T-160	40,500	15,000	3.0	4.7
T-2-56	20,750	10,000	3.0	4.4
XT-12-58	33,000	9,750	2.6	4.1
XT-Ta-14-58	41,000	15,000	2.5	3.9
XT-TA-10-58	40,750	16,750	2.8	4.4

Both growth and number of stems per acre were subjected to an analysis of variance. No significant differences were found between materials or location within the trial. The number of stems greater than 3 feet in height was more consistent. Suckering clearly was sufficient to occupy the site within one year of harvesting.

XPERIMENTAL TRIAL LIII

The current interest by the U.S. Forest Service, U.S. Department of nergy, and a number of other organizations in the utilization of black poplars nd black poplar hybrids for "biomass" production prompted us to measure a clo- al trial established as part of Project 1800 in May, 1970. Black poplars and alsam poplars were included as part of the Institute's early work with the genus opulus. A number of controlled crosses with IPC parent tree selections were ade and field tested. Along with the seedling work, clones were selected by PC and also acquired from other organizations. Trial LIII is one of the clonal ests used in the projects' evaluation of black poplars.

The following information was gathered for a paper presented at the th North Central Tree Improvement Conference held in Fargo, North Dakota in ugust, 1987 (Wyckoff et al., 1987). The information is included in this report o provide an indication of the suitability of these materials when grown under he conditions described.

Stem cuttings of 38 clones were rooted and grown for one year at the PC nursery near Greenville, WI. Thirty-six clones were provided by Dr. John erbee from the University of Wisconsin Arboretum, and two were IPC selections Table 5). The trial, established in 1970 on a typical cottonwood site Popovich 1982, Barkley 1983) near Appleton, WI, consisted of a randomized block esign with four replications. Each clone was represented by two rows of four uttings in each replication. Spacing was 8 ft by 12 ft. Variation in cutting vailability and quality limited the number of replications for some clones and revented establishment of border rows around individual replications and around he entire planting.

Table 5. Clone parentage and source.

Clone Identification	Source	Parentage
W-2	University of Wisconsin	P. deltoides
W-5		P. deltoides
W-105		P. deltoides
W-117		P. deltoides
W-126		P. deltoides
W-132		P. deltoides
W-136		P. deltoides
W-140		P. deltoides
W-1045		P. deltoides
W-1070		P. deltoides
H-416-F	Harvard University	P. deltoides
H-424		P. deltoides
H-551		P. deltoides
H-585		P. deltoides
H-665		P. deltoides
H-778		P. deltoides
H-1076		P. deltoides
H-1109		P. deltoides
H-1633		P. deltoides
H-1651		P. deltoides
*W-58		P. deltoides cv virginiana x P. balsamifera
NE-32	Northeast Forest Experiment Station	P. angulata x P. berolinensis
NE-35		P. angulata x P. platierensis
NE-225		P. deltoides cv 'virginiana' x P. caudina
NE-238		P. deltoides cv virginiana x P. nigra cv 'volga'
NE-244		P. angulata x P. deltoides cv 'virginiana'
NE-265		P. angulata x P. nigra cv 'volga'
C-4	Ontario Department of Lands & Forests, Maple Ontario	P. x euramericana cv 'betulifolia'
C-5		P. x euramericana cv 'robusta'
C-14		P. x euramericana cv 'erecta'
IHAM, C-444		P. x canadensis
IH-78B, C-445		P. x euramericana cv 'Jacometti 78B'
IH-30A, C-450		P. x canadensis
*W-85		P. x canadensis cv 'charkowiensis'
*W-87		P. x canadensis cv 'charkowiensis'
C-401		P. x euramericana cv 'gelrica'
DH-4-62	Institute of Paper Chemistry	P. x euramericana
DH-9-62	Ontario Paper Co. & Institute of Paper Chemistry	P. x euramericana cv 'robusta raverdeau'

*Dr. John Berbee's number, University of Wisconsin.

Soils at the site are in the Manawa Series, which are fine, mixed, sic Aquollic Hapludalfs (Barndt et al. 1978), and best described as silty clay ams with high fertility and moderate organic matter content (Table 6). infall averages 30.8 inches annually, with 20.7 inches occurring between ril and September.

Replications were arranged to account for apparent slope and soil isture gradients. Two replications were located on the higher and somewhat ier portion of the site, another on the more sloping section, and the last at e lower, flatter, and wetter end of the trial.

ble 6. Soil texture and nutrient analysis.

mple pth	pH	Organic Matter, tons/acre	Lbs/Acre N	P	K	Ca	Mg	Texture
6"	6.7	60	4282	44	180	9500	1400	Silt loam
12"	6.8	34	2428	20	175	7500	1400	Silty clay loam
-18"	6.8	18	1286	9	215	6650	1500	Clay

The site had been planted to corn for the two seasons before planting. te preparation consisted of disking, and the rooted cuttings were hand-planted. intenance involved rototilling the first three years after planting and riodic mowing thereafter.

Survival, general condition, and total height were measured 1, 3, 5, , and 17 years after planting. DBH was measured at the end of the third owing season and in each measurement year thereafter. Bole cankers caused by ptoria musiva and Fusarium solani were first observed during the fifth growing

season, and incidence was recorded in years 10 and 17. Relative volumes were computed as D^2H to reflect potential productivity at 10 and 17 years.

Imbalance prevented meaningful statistical analyses of all 38 clones. To quantify clonal differences and genetic parameters, analyses of variance therefore involved only those 20 clones that were adequately represented in three replications. Data from the lowest lying replication were excluded.

Analyses of growth parameters were also restricted to those four trees per clone per replication that were tallest at age 17. This method was adopted to offset the effects that cutting and rooting quality had on early growth of many ramets and clones and more realistically represent the potential of individual clones. The goal was to evaluate a representative sample of material that most likely was all in good and equal condition at planting. Some upward bias undoubtedly occurred as a result, but rather few measurement trees were from the edge of the planting or from openings within it. Relative differences among clones thus appear quite realistic.

Analyses of survival data, in contrast, were based on all trees alive at each measurement date, and those of disease incidence include all trees ever cankered, whether dead or alive.

An analysis of variance (ANOVA) for randomized blocks with subsamples (the model assumed fixed replication and clone effects) was used to evaluate differences between clones and calculate variances required for heritability estimates.

Broad sense heritability estimates were computed by comparing variation among clones (genetic component) with that within clones (environmental component).

Overall survival (Table 7) averaged 86 percent at the end of the 10th owing season. Several clones, e.g., H-1633, proved difficult to establish and d considerable early mortality, clearly reflecting the problems posed by tting and rooting quality. By age 17, average survival had fallen to 67 per- nt, largely as a result of the dramatic increase in canker incidence. Most ees dying during this period were cankered, and many had been cankered at age).

Variation among clones in terms of survival was significant (data not own), and several survived and grew well despite large increases in canker ncidence. W-1070, the fastest growing clone according to the analyses of ariance, averaged 96 percent survival at 10 years and lost only four percentage oints between years 10 and 17 (Table 7). Its canker incidence, in contrast, ose from zero to 68 percent. Replication effects on survival were non- ignificant.

Variation in growth traits was considerable over all clones (Table 7), nd significant among those examined in analyses of variance (Table 8). Despite he significant variability, few clones proved particularly better or worse than thers. Height of the majority at 17 years exceeded 50 ft, and DBH typically as greater than 7 inches. Annual height growth averaged 3.7 ft through the 0th growing season but dropped to 2.9 ft between ages 10 and 17. Similar pat- erns were noted for DBH and D_2H. Some portion of the decrease in annual growth ates can be explained by crown closure, but the considerable increase in canker ncidence undoubtedly also played a role.

The fastest growing and most productive clone, W-1070 (Fig. 3), was of ure P. deltoides parentage (Tables 5 and 8). The slowest growing clone, W-58,

Table 7. Overall survival, growth, and canker incidence of P. deltoides and hybrid poplar clones.

	Age 10				Age 17			
Clone	Total Ht. Ft.	DBH, inches	% Bole Canker	% Survival	Total Ht. Ft.	DBH, inches	% Bole Canker	% Survival
IH-30A	46	5.9	6	100	58	8.0	100	94
W-1070[1]	42	5.3	0	96	58	7.4	68	92
W-132	44	5.6	10	91	57	7.6	86	84
C-5	41	5.2	10	90	57	7.3	89	88
NE-238	41	5.6	23	97	56	7.6	96	53
W-87	33	5.6	3	94	56	7.4	96	91
W-1045	42	5.2	12	100	56	7.0	90	88
C-401	40	4.9	7	91	55	7.8	82	47
H-1076	43	5.5	0	97	55	7.4	97	97
W-136	41	5.1	0	94	55	7.0	96	91
H-585	41	4.9	3	94	55	6.9	96	88
NE-265	37	4.0	0	94	55	6.6	37	85
IH-78B	42	5.9	0	97	54	8.1	46	72
DH-9-62	44	5.7	19	97	54	7.5	92	59
DH-4-62	44	5.3	6	100	54	7.0	100	100
W-5	39	5.6	7	94	53	8.0	83	91
H-1651	40	5.5	0	88	53	7.7	100	82
W-117	39	4.7	0	97	53	6.8	79	91
W-2	38	4.6	0	69	53	6.6	58	60
H-665	38	5.0	12	79	51	7.4	100	78
NE-225[1]	36	5.0	28	84	51	6.9	94	54
W-140[2]	38	4.2	0	82	51	5.8	100	68
IHAM	35	4.4	24	78	50	7.3	64	44
NE-35	37	4.8	12	100	49	6.4	97	97
W-126[1]	34	3.8	0	75	49	5.4	69	67
NE-32[1]	35	4.5	14	92	47	6.7	100	58
W-105[3]	30	3.2	0	75	46	5.6	100	62
H-778	33	4.1	0	92	45	5.5	0	63
C-4[3]	32	3.6	0	50	44	6.0	100	50
H-551	34	5.1	0	78	42	6.6	32	69
W-58	32	4.9	100	91	39	6.2	100	75
NE-244[2]	30	3.6	0	68	39	5.6	0	56
W-85	32	4.1	7	91	38	7.1	100	12
H-1109[3]	24	3.6	0	50	37	4.8	100	38
H-424[3]	32	3.5	0	100	37	4.1	0	75
H-1633[3]	17	2.3	0	50	27	3.4	0	25
H-416F[2]	25	3.3	0	63	--	--	0	0
C-14[1]	40	4.6	56	96	--	--	100	0

No superscript indicates data are averages of four replications.
[1]Data are averages of three replications.
[2]Data are averages of two replications.
[3]Data are averages of one replication.

able 8. Means for growth traits at age 17 and canker incidence at ages 10 and 17 for the 20 clones examined in analyses of variance.[1]

	Age 17				Age 10
Clone	Height, feet	DBH, inches	D^2H	% Bole Canker	% Bole Canker
-1070	62[a]	8.3[abc]	4274[a]	70[a]	0[c]
-87	59[ab]	7.8[abcde]	3661[abcde]	95[a]	4[bc]
H-30A	58[ab]	7.4[bcdefg]	3214[abcdefg]	100[a]	4[bc]
-132	57[ab]	8.2[abcd]	3829[abcd]	87[a]	4[bc]
H-4-62	57[ab]	7.6[abcdef]	3278[abcdefg]	100[a]	4[bc]
-5	57[ab]	7.8[abcde]	3514[abcdef]	87[a]	0[c]
-1076	57[ab]	7.8[abcde]	3596[abcde]	96[a]	0[c]
-136	56[abc]	7.4[bcdefg]	3048[bcdefg]	94[a]	0[c]
-585	56[abc]	7.0[defg]	2746[defgh]	95[a]	4[bc]
-1045	55[abc]	7.1[cdefg]	2811[defg]	84[a]	12[b]
E-265	55[abc]	6.8[efg]	2617[defgh]	24[b]	0[c]
-1651	55[abc]	7.3[bcdefg]	2876[cdefg]	100[a]	0[c]
-5	55[abc]	7.6[abcdef]	3223[abcdefg]	79[a]	8[bc]
-665	54[bcd]	8.6[ab]	4090[ab]	100[a]	4[bc]
-117	54[bcd]	6.7[efg]	2450[efgh]	71[a]	0[c]
H-78B	54[bcd]	8.7[a]	4042[abc]	25[b]	0[c]
-2	52[bcd]	6.4[fg]	2400[fgh]	67[a]	0[c]
E-35	49[cd]	6.9[efg]	2375[fgh]	100[a]	8[bc]
E-32	48[d]	6.5[fg]	2102[gh]	100[a]	13[b]
-58	40[e]	6.3[g]	1605[h]	100[a]	100[a]

[1] Comparison of 4 tallest trees per replication from clones with 3 replications with the exception of bole canker where all trees were used.

bc...Duncan's New Multiple Range Test was calculated when "F" test values for treatments were significant. Values followed by a common superscript letter are not significantly different.

was a hybrid cross involving P. balsamifera, and was the most severely cankered entry at both 10 and 17 years. Aside from a few observations such as the above, no clear or consistent pattern between parentage and growth was apparent.

Figure 3. A 17-year-old hybrid poplar clone, W-1070, growing on the Greenville Test area in Trial LIII. It averaged 58 feet in height and 7.4 inches in diameter. The ramets shown are in a replication with favorable growth conditions and are larger than the same individuals in other replications. Black poplars are sensitive to site quality and to get the best growth must be placed on suitable sites.

Replication effects on growth traits were also significant. Height owth was lower in replications at the higher and drier end of the planting 3 ft and 52 ft versus 59 ft), suggesting that many clones of the type included i this trial are sensitive to site conditions and perhaps not well-suited to ›re upland areas.

The number of pathogens (Septoria, Fusarium, Cytospora) responsible for ıe many bole cankers in this trial indicates that disease resistance must be ›nsidered in selection, breeding, and culture. The dramatic increase in inci- ence between ages 10 and 17 also underscores the need for long term testing. ›st clones had little or no infection at age 10, but the greater majority had) or more percent infection at 17 years (Tables 7 and 8). Numerous cankers ccurred in the upper portions of crowns and contributed to death and/or reakage of both branches and tops.

Variation among clones evaluated in analyses of variance was signifi- ant, with the best two being responsible for much of the variation (Table 8). hese clones NE-265 and IH-78B, were both hybrids, with only one containing P. eltoides germplasm. In contrast, most clones having 90 percent or more infec- ion after 17 growing seasons had P. deltoides in their pedigree. The most usceptible clone, W-58, was a P. deltoides x P. balsamifera hybrid, and exten- ive cankering undoubtedly contributed to the slow growth of this and similar lones. The impact of disease incidence on product quality and value are less asily determined, but may be as significant as or more so than those on urvival and growth. Holt et al. (1981), for example, found that pulps from ankered trees had higher lignin content, more extractives, and less strength han those from trees free of disease.

Broad sense heritability estimates, as determined under the conditions of this experiment, indicate that height growth is under moderate to high genetic control (Table 9). A similar value was reported by Wilkinson (1973). Those for diameter growth were somewhat lower, but nevertheless sizeable and similar across ages. The estimates for D^2H at both ages 10 and 17 suggest that concurrent selection for height and DBH would lead to significant gains in volume productivity. Bole canker infection at age 17 also appears to be under moderate genetic control. The rather large estimate at age 10 (0.93) is inflated and unrealistic as a result of low infection levels. Variation among clones was maximal (0 to 100 percent), while that caused by other sources, especially within clones, was minimal. Such findings confirm that long term testing and uniform, heavy infection are essential for reliable estimates of clonal worth and genetic parameters.

Phenotypic correlations among growth traits were quite strong within and across ages (Table 10). Thus, the fastest growing clones at age 10 were among the tallest and most productive after 17 growing seasons. In addition, no relationship was found between growth at 10 years and disease incidence at either 10 or 17 years. The fastest growing clones were not necessarily more or less susceptible than others.

In sum, survival and growth of several clones in this experiment proved quite good, and a few of them may be useful for intensive culture and/or future breeding. Widespread susceptibility to cankering, however, indicates that disease must be considered in designing tests and making selections. The site sensitivity of such materials was demonstrated by significant growth differences among replications. Thus, expected volume productivity and gains therein can be achieved only by close matching of planting material to site.

ble 9. Broad sense heritabilities for growth traits and canker incidence.[a]

Parameters	Age 10	Age 17
Total height (H)	0.31	0.46
DBH (D)	0.24	0.26
D^2H	0.43	0.42
% Trees with bole cankers	0.93[b]	0.39[b]

[a]Estimates based upon 20 clones, 3 replications, and 4 best trees per replication, except as noted.
[b]Estimate based upon 20 clones, 3 replications per clone and replication averages.

ıble 10. Simple phenotypic correlations among traits within and across ages.

Factors	DBH Age 10	Height Age 10	D^2H Age 10	DBH Age 17	Height Age 17	D^2H Age 17	Canker, % Age 17
BH - age 10	1	0.719	0.974	0.785	0.650	0.311	0.093
t. - age 10		1	0.818	0.457	0.758	0.353	0.104
^2H - age 10			1	0.744	0.710	0.335	0.117
BH - age 17				1	0.702	0.399	0.136
t. - age 17					1	0.406	0.123
^2H - age 17						1	0.108
anker % - age 17							1

orrelation coefficients - n = 96, $r_{0.05} = 0.203$, $r_{0.01} = 0.263$.

TRIAL LXVII - MICHIGAN DEPARTMENT OF NATURAL RESOURCES

A small trial testing five triploid hybrid aspen clones and three hybrid aspen families was planted in May, 1987 on Michigan DNR land in Charlevoix county in northern Lower Michigan. The trial is composed of 25 blocks of 25 trees planted in a completely random design. Insufficient numbers of ramets for each of the clones prevented the use of a randomized block design.

The trial is on a northern hardwood site that had been clearcut, but areas of standing timber remained at the time of planting. Slash was evenly distributed and made hand planting difficult. Soils are loamy sands. Unfortunately, observations later in the summer indicated that the logger returned after the trial had been planted and ran a skid trail across the SE corner of the trial. In addition, the standing timber near the trial was cut and dropped directly into the trial.

Because of the damage and the travel time required to reach the trial, it was not measured this fall. It will be observed this coming summer, and if warranted, measured next fall.

TRIAL LXVIII - CONSOLIDATED PAPERS, INC.

A small, four replication, randomized block trial testing three triploid hybrid clones and three triploid hybrid families was planted on Consolidated Papers' land near Loretta, Wisconsin. One of the soil samples described in Tables 13 and 14 for the Easterhouse Road planting discussed on page 35 was taken from Trial LXVIII. The three crosses in the trial are also in the surrounding 32 acre planting and will provide additional growth information. The three clones are selections from replicated trials on the Ripco Test Area and a silvicultural trial in Monico, Wisconsin.

The clones were selected for their rapid growth and form. The trial ill be part of the testing required to determine if they are indeed superior to ybrid seedlings. First year growth and survival are given in Table 11. One lone, XT-Ta-6-64,S-1, was in poor condition at the time of planting. Roots and tems on a number of individuals had blackened and had to be discarded or runed. Several of the ramets died soon after planting, and the majority of the urvivors had poor growth. Growth and survival of the other five materials was ;ood.

'able 11. First year growth and survival summary of Trial LXVIII.

Material[a]	Av. Ht. Ft.	Survival, %
XT-Ta-10-58,S-5	2.3	93
XT-Ta-6-64, S-1[b]	1.5	48
XT-Ta-10-69, S-1[b]	2.5	88
XT-Ta-5-86	2.4	93
XT-Ta-11-86	2.8	98
XT-Ta-13-86	2.6	98

[a]See appendix for description of cross number.
[b]Means clone, not seedlings.

DEMONSTRATION PLANTING EVALUATION

CONSOLIDATED PAPERS, INC. - EASTERHOUSE ROAD PLANTING

A joint proposal was submitted to the Department of Energy in 1985 to evaluate the feasibility of using hybrid aspen conversion plantings for "biomass" production. To prepare for the possible acceptance of that proposal, a site was selected, harvesting was arranged, and stock was grown. Unfortunately, the proposal was not accepted, but the planting went forward.

The site was whole tree harvested the fall of 1986 and spring of 1987. Both roundwood and chips from unmerchantable material were produced. A summary of the wood removed from the 76-acre, medium quality hardwood clearcut is given in Table 12. The age of the stand was 85 years with a site index of 60. Soils had silt loam textures in the upper 18 inches (Table 13) and soil nutrients were good (Table 14 and Fig. 2).

Table 12. Wood and residue removal from Easterhouse Road conversion site.

Species	Pulpwood Cords	Boltwood Cords
Mixed hardwoods	1506	58
Balsam fir	36	--
Aspen	27	--
Hemlock	21	--
White pine	12	--
Unmerchantable (Hdwd. & conifer)	Chips Cords	Chips Green Tons
	793	1824

As can be seen from the volume data given in Table 12, the site produced approximately 31.5 cords per acre including tops and unmerchantable wood.

nual production appears to have averaged only about 0.4 cords per acre. The and components and soil quality indicate the site will be a productive one for brid aspen, perhaps yielding two cords per acre per year.

ble 13. Soil texture analysis of Consolidated Papers Easterhouse Road.

Planting Sample Number	Sample Depth, inches	Sand, %	Silt, %	Clay, %	Texture Class
1	0-6	30.0	52.6	17.4	silt loam
	6-12	24.2	59.4	16.4	silt loam
	12-18	30.0	56.8	13.2	silt loam
2	0-6	36.4	53.0	20.6	silt loam
	6-12	26.2	56.2	17.6	silt loam
	12-18	28.4	56.0	15.6	silt loam
3	0-6	22.4	60.0	17.6	silt loam
	6-12	25.6	61.4	13.0	silt loam
	12-18	24.2	62.6	13.2	silt loam

able 14. Soil nutrient analysis of Consolidated Papers Easterhouse Road planting.

ample umber	Sample Depth	pH	Organic Matter t/A	lb/acre N	P	K	Ca	Mg
1	0-6	4.6	37	2643	15	55	800	180
	6-12	4.8	23	1643	20	40	400	110
	12-18	4.9	6	428	11	45	850	340
2	0-6	4.6	26	1857	24	65	950	180
	6-12	4.8	19	1357	16	60	600	140
	12-18	5.0	14	1000	10	40	550	150
3	0-6	4.8	20	1428	10	40	1000	150
	6-12	5.0	11	786	8	35	900	140
	12-18	5.1	3	214	11	35	750	230

Thirteen thousand two hundred bareroot triploid hybrids from four crosses were hand planted by a professional planting crew on May 21-24, 1987. Thirty-eight 1/25 acre growth plots were taken after the first growing season. Average height for all three sources was 2.1 feet with best stems over 5 feet (Fig. 4). The target density of 500 trees per acre was met in several areas of the planting but overall stocking was 406 stems per acre. First year mortality appears to be less than 1% - one dead seedling was found in one of 38 plots tak

Figure 4. First year's growth on triploid hybrid aspen in Consolidated Papers Easterhouse Road planting. Overall height growth average was 2.1 feet with survival greater than 95%. Areas of better than average growth, as shown above, were noted across the plantation.

The stock had been root pruned at the time of lifting, and discussions with the planters indicated they had no problems planting it properly. Had the stock not been pruned planting would have been difficult.

There was no site preparation. The major competition problem was climbing false buckwheat which developed extensive mats of vegetation. In a number of instances, seedlings were entwined so tightly they were almost girdled (Fig. 5). Additional physical damage occurred when the vegetation bent the seedlings over and held them, causing a bud to break and a new shoot to establish apical dominance. False buckwheat probably will be less abundant and troublesome this coming growing season. Deer browsing has been more extensive than expected, and availability of other browse material should prevent serious damage.

CONSOLIDATED PAPERS, INC. - MOOSE LAKE PLANTING

The original intent was to establish 20,000 seedlings on the Easterhouse Road site, but harvesting had been completed on only 30 acres and was continuing at the time of planting. A second site was then selected and planted with 7300 seedlings from one triploid cross (XT-Ta-5-86) by the same planting crew. The Moose Lake site was also a hardwood clearcut but had been prepared for planting with a disk trencher. Though not sampled, the soil appeared to be sandy loam, with abundant large rock. Observations of the residual stand and comments by the area forester indicated the site quality was better than that of the Easterhouse Road site.

A series of 13, 1/25 acre plots were taken at the end of the first growing season. Average height growth was three feet, ranging from 1.5 feet to over 9 feet (Fig. 6). Average stocking was 631 trees per acre. The caliper and apparent vigor of even the smaller stems indicates successful establishment. As with the Easterhouse Road planting, only one dead seedling was found in the 13 plots. Climbing false buckwheat was extensive, but was not as damaging as on

the Easterhouse Road site. Only minor deer browsing occurred, but pressure may develop. If damage does occur, size and vigor should ensure quick recovery.

Figure 5. Climbing false buckwheat covered extensive areas of the Easterhouse Road and Moose Lake plantings and is common on many Lake States sites. As can be noted, the climbing vine has almost girdled the planted hybrid. In other cases, the vine has bent seedlings over and slowed growth.

Figure 6. A large number of triploid hybrid aspen on Consolidated Papers' Moose Lake planting have put on excellent first year height growth. The best stems were over 9 feet. Plantation survival was over 95%.

CLONAL SELECTION

The use of vegetatively propagated superior individuals for reforestation can result in significant gains in fiber production, wood quality, site adaptability, and disease resistance. Much of the eucalyptus fiber imported into the United States is from plantations established with selected clones. Uniform wood quality, growth rate, and stem size at harvest are among the advantages. Southern tree improvement programs are also giving increased attention to clonal forestry, considering methods to propagate both hardwoods and softwoods.

Many species such as poplars, willows, some spruces, and eucalyptus can be propagated with relative ease from cuttings. Tissue culture holds promise as a method to rapidly produce large numbers of selected genotypes that would be difficult to propagate by other means.

The aspen project has been acquiring selections from a number of test plantings within the Lake States. These selections are being propagated from root sprouts and will be evaluated in a series of clonal trials, two of which will be planted this coming spring. A listing of this year's root sprout production can be found in Table 3.

1987 HYBRID ASPEN SELECTIONS

Twenty-two selections were made this past year, including an exceptional seedbed selection and a two-year-old individual from a plantation. All but three are diploids and represent P. tremuloides, P. grandidentata, P. tremula, and P. davidiana parentage. Table 15 describes the 1987 selections. Figure 7 illustrates a natural hybrid clone and a clonal selection from a twenty year-old planting.

Table 15. 1987 Clonal selections.

Clone Identification[a]	Location[b]	Age	Total Ht., ft.	DBH, inches
XT-Ta-60-60,S-1	Trial XVI	26	77	12.5
XT-Ta-61-60,S-1	Cl. Rep. Misc.	26	96	15.0
XT-Ta-64-60,S-2	Trial XVI	26	81	12.2
XT-Ta-65-60,S-3	R. Rep. Misc.	26	78	12.0
XT-Ta-65-60,S-4	R. Rep. Misc.	26	89	13.8
XT-Ta-65-60,S-5	R. Rep. Misc.	26	81	13.8
XT-Da-18-59,S-11	R. Rep. Misc.	27	69	13.9
XT-Da-18-59,S-12	Trial XII	22	77	10.8
XT-Da-18-59,S-13	Trial XII	22	74	10.3
XT-Da-18-59,S-14	Trial XII	22	68	10.2
XT-Da-18-59,S-15	Trial XII	22	73	9.9
XT-Da-30-59,S-1	Trial XII	22	74	9.3
XG-Da-26-59,S-2	Trial XII	22	72	8.6
XG-Da-26-59,S-3	Trial XII	22	78	9.8
XG-Da-26-59,S-4	Trial XII	22	71	7.2
XG-Da-26-59,S-5	Trial XII	22	77	8.6
XG-Da-26-59,S-6	Trial XII	22	78	10.0
XCa-G-37-67,S-2	Cl. Rep. Misc.	20	75	18.2
XG-Ta-13-66,S-1	Cl. Rep. Misc.	21	71	17.9
XG-Ta-14-66,S-1	Cl. Rep. Misc.	21	71	14.2
XT-Ta 3n	Blandin Plntg.	3	15	1.4
XCa-O-27-86,S-1	IPC Seedbed	1	5.5	--
AG-4-66	Seymour, WI	78	103	32.1

[a]See Appendix for description of identification code.
[b]Cl. = Clintonville Test Area; R. = Ripco Test Area; Rep. Misc. = Replicated Miscellaneous.

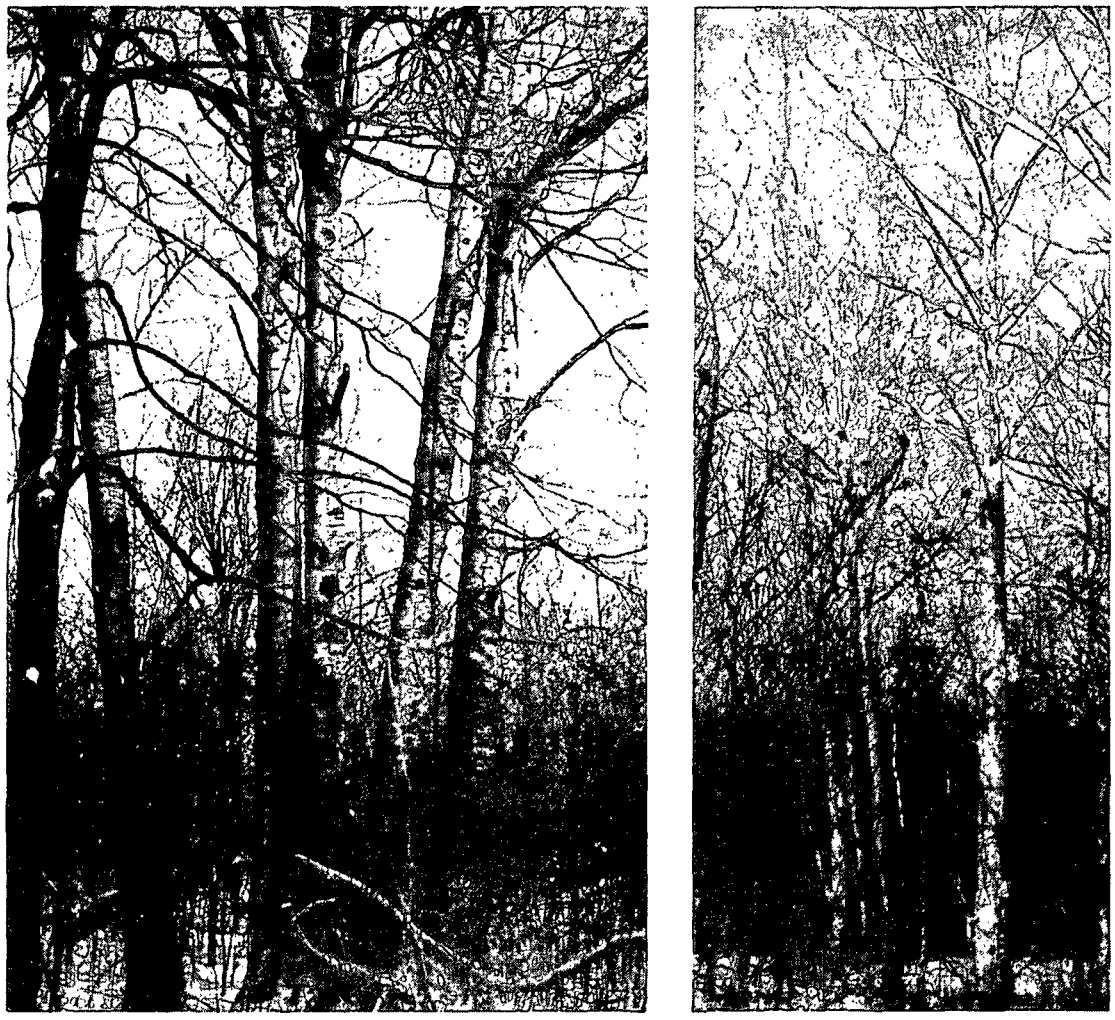

Figure 7. The clonal selection on the left (AG-4-66) is a natural hybrid between Populus alba and P. grandidentata discovered in 1966. It is now 78 years of age. The five stems shown range in diameter from 22.2 inches to 32.1 inches with heights up to 90 feet. The clonal selection on the right is from a 20-year-old P. canescens x P. grandidentata cross, XCa-G-37-67 growing on the Clintonville Test Area. The selection is 75 feet in height and 18.2 inches dbh.

The 1986 and 1987 selections represent diverse types of aspen hybrids. Most have been selected for growth characteristics, but others such as the P. tremuloides x P. davidiana and P. canescens x P. grandidentata were selected because of their apparent resistance to the bronze leaf disease as well as

growth. Roots have been collected from all of the 1987 selections and are being stored for use in late February. As with the 1986 selections, ramets from each clone will be propagated for testing.

PROPAGATION OF SELECTED CLONES

Fifteen hybrid selections made in 1986 were propagated from roots during the winter of 1987. As noted in Table 16, the approximate length and diameter of the roots started from each clone and the number of sprouts produced were recorded and provide an indication of production requirements. It is important to note that the number of sprouts produced is for approximately a 10 week period. Sprouts were still being produced, but the time restriction for rooting and transferring to the nursery dictated the cutoff time for further production.

The best sprout production occurred from roots less than 1/4 inch in diameter. The first sprouts were removed 14-21 days after the roots were placed in the greenhouse. Rooting success varied by clone but appeared to be related to cultural conditions. Although all roots were surface sterilized and cuttings handled in a clean manner, pathogens were more evident in some clones than others. Subsequent rooting work with tissue culture derived plants under controlled conditions, particularly humidity, indicates a much higher rooting rate is possible.

A sufficient number of ramets per clone are available for testing this coming spring. Two replicated trials will be planted, one near Grand Rapids, Minnesota, on Blandin Paper Co. land, and one near Appleton, Wisconsin, on land near the IPC Greenville Test Areas.

Table 16. Rootsprout production.

Clone	Length of Root, ft.	Av. Root Diam., inches	No. Sprouts Produced	Sprouts/ Ft. of Root
XT-Ta-10-69,S-1	90	< 3/8	1093	12
XT-Ta-10-69,S-1	59	< 1/4	632	11
XT-Ta-10-58,S-5	93	< 1/4	950	10
XT-Ta-14-58,S-3	27	< 1/4	270	10
XT-Ta-14-58,S-23	50	1/4-5/8	488	10
XT-Ta-14-58,S-24	30	1/4-1/2	90	3
XT-Ta-14-58,S-25	14	3/8-5/8	95	7
XT-Ta-14-58,S-26	11	1/8-3/4	107	10
XT-Ta-14-58,S-27	13	1/8-5/8	133	10
XT-Ta-14-58,S-28	9	1/2-3/4	65	7
XT-Ta-14-58,S-29	13	3/8-3/4	145	11
XCa-O-27-86,S-1	10	< 3/8	141	14
XT-Ta-6-64,S-1	282	< 3/8	713	2
XCa-G-38-67,S-1	13	< 1/4	32	3
XCa-G-38-67,S-2	44	< 1/4	266	6

TISSUE CULTURE PROPAGATION OF ASPEN

In a clonal forestry program, a suitable method for large-scale, economical vegetative propagation is required. In aspen, production of root sprouts from root segments currently is the most effective method of vegetative propagation. However, *in vitro* propagation from shoot cultures also holds promise, if competitive with root sprout production. To compare conventional and *in vitro* vegetative propagation, shoot cultures were established from 13 clones

selected for clonal trials. Early (3 month) evaluation of the cultures revealed that the majority (7 of 13) of clones readily form shoot cultures. Of the remaining clones, only one was unresponsive in culture.

Small cuttings (12-24 inches) containing dormant lateral buds were harvested in November. Cuttings were obtained from rooted root sprouts lifted from nursery beds. Buds were picked from the cuttings and washed for 30 minutes in a solution containing a small amount of household dishwashing soap (Palmolive). Buds were sterilized with 10% commercial household bleach (Hilex, 0.525%) for 15 minutes. After three rinses with sterile water, the shoot tips (plus 3-4 primordial leaves) were excised and sterilized with 1% Hilex for five minutes. After three additional rinses with sterile water, shoot tips were placed on WPM medium (Lloyd and McCown, 1980) containing 0.05 mg/L NAA and 1.0 mg/L BA solidified with 0.8% agar (pH = 5.6). Following the procedure of Sutter and Barker (1986), shoot tips were transferred to fresh medium (by simply moving the bud to a different part of the Petri dish) every 3-4 days. After 2-3 months the clones were evaluated on the basis of the extent of multiple shoot production.

The ability to produce multiple buds <u>in vitro</u> in the 13 clones examined is presented qualitatively in Table 17. Clones described as "rapid" means that within 3 months, the initial shoot tip rapidly elongated and either (1) formed adventitous shoots, or (2) exhibited elongation of axillary buds on the initial shoot. Some shoots in these cultures had reached several centimeters in length by this time. Clones under the category of "slow" means that shoot elongation was at an early stage after 3 months. Such clones could be "rapid," given enough time and/or minor adjustments in the composition of the medium. One clone, XT-Ta-14-58, S-28 was unresponsive. Extensive adjustment of culture conditions may be required in order to establish shoot cultures from this clone.

Table 17. Evaluation of the potential for multiple shoot formation *in vitro* in 13 aspen clones.

Multiple Shoot Forming Potential		
Rapid	Slow	Unresponsive
XT-Ta-14-58, S-29	XT-Ta-10-69, S-2	XT-Ta-14-58, S-28
XT-Ta-14-58, S-27	XT-Ta-14-58, S-3	
XCa-G-38-67, S-1	XT-Ta-14-58, S-24	
XT-Ta-14-58, S-23	XT-Ta-14-58, S-25	
XCa-G-38-67, S-2	XT-Ta-14-58, S-26	
XT-Ta-6-64, S-1		
XT-Ta-10-69, S-1		

It is of interest to note that the best clone, XT-Ta-14-58, S-29 and the worst, XT-Ta-14-58, S-28 are from the same full-sib family. This is not altogether unexpected as in many instances intrafamily variation often is as great or greater than variation between families or even between species. However, it should be noted that this apparent genetic variation may not be absolute. As noted, changing the culture conditions could increase efficiency. In particular, it appears that *P. canescens* x *P. grandidentata* clones might require lower levels of cytokinin.

The method chosen to produce shoot cultures was that developed for *in vitro* propagation of a tetraploid European aspen clone (Wann *et al.* 1987), it seems suitable for propagation of these 13 clones. Owing to the availability o root material from these clones, a fair assessment of the economics of tissue culture *vs.* conventional propagation system should be possible.

HYPOXYLON RESEARCH

A rapid, inexpensive method for early testing and selection of aspen resistant to Hypoxylon mammatum would be most useful. Toward this end, we continued work with the leaf bioassay, much as described in earlier reports (see Report Two, p. 38-46). One additional progeny group and 17 clones were evaluated in 1987. Thirteen of the clones were hybrids, all of which were considered resistant on the basis of the leaf bioassay. Three other clones were P. tremuloides selections, and all gave susceptible responses. The remaining two gave intermediate reactions.

Some further work was done on the relationship between responses in the leaf bioassay and the natural course of Hypoxylon incidence and mortality in field trials. Relationships between incidence and other variables, including mortality, in the field was also examined. Four families, used in past bioassay work, were checked in preliminary analyses. As expected, trends in mortality generally parallel those for incidence but are offset in time by several or more years. This parallelism resembles that noted in other forest tree diseases and may provide a basis for early prediction of mortality at the end of the rotation in both test and operational plantings.

A strong and linear relationship was found between incidence 10 and 15 years after planting and mortality at age 20 years. Correlation coefficients were not statistically significant - an expected outcome in that only the means for four families were used. Inclusion of replication means and/or more families is expected to yield significant correlations and perhaps regression equations of predictive value. Understanding such relationships may also shed light on both degrees and types of resistance, thereby allowing us to separate

resistant from susceptible individuals and families faster and more accurately. As an example, many trees in the most "resistant" family remained free of infection over the 20-year period, but most infected individuals died soon after noticeable infection.

Results of these analyses and those of additional families will guide future bioassay work. Given an encouraging outcome, we may collect and use Hypoxylon isolates from roughly 10 trees, ranging from resistant to susceptible in field trials. These would be contrasted with one another and a "wild-type" from an adjacent native stand to determine if any of the excessive variation noted to date results from differences in the fungus. They will also be used to evaluate utility of a second bioassay method, insertion of mycelia into the stems of young seedlings. This approach was developed in France to screen for resistance in P. tremuloides and tremula. Its originator, Dr. Pinon, visited us last summer, explained the technique, and encouraged us to try it. The method appears attractive in that the infection court and inoculation procedure are more like the natural process than the leaf bioassay.

PLANS FOR 1988/89

Seed production and methods to improve current seed use will continue to be a major part of the aspen project. Additional support for this aspect of the project is being sought. A proposal will be prepared and presented to prospective cooperators. Clonal propagation and testing of hybrid aspen selections will receive increased attention. The gains in volume production from clonal selections in other species have been clearly demonstrated, and similar gains are expected with hybrid aspen.

Operational scale plantings will be closely followed to aid in providing guidelines for future plantings. Field trials and plantings pertinent to current hybrid aspen work will be measured. A field meeting in conjunction with the larch project will be held in late summer, 1988, in Northern Wisconsin and Northern Minnesota.

An aspen geneticist from China visited IPC for three weeks this past summer and discussed his work and potential areas of collaboration. It was agreed that we would exchange pollen this winter and produce a number of small crosses. Seed from those crosses will be exchanged and seedlings grown for testing. Of particular interest was a cross between our P. tremuloides and China's P. tomentosa, an aspen similar to our P. alba.

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LITERATURE CITED

Barkley, B. A. 1983. A silvicultural guide for hybrid poplar in Ontario. Ministry of Natural Resources, Ontario. 35 p.

Barndt, W. D., Lorenz, H. E., and Frings, S. W. 1978. Soil survey of Outagamie County, Wisconsin. USDA Soil Conservation Service. 129 p.

Bolt, K. E., McNabb, H. S., Jr., Rothlauf, M. A., Manwiller, F. G., and Ostry, M. E. 1981. Characteristics of kraft paper made from Septoria cankered Populus grown under short-rotation, intensive culture. Abstract, Phytopathology 71, No. 2, p. 226.

Lloyd, G. and B. H. McCown. 1980. Commercially feasible micropropagation of mountain laurel (Kalmia latifola) by use of shoot tip culture. Combined Proc. of the Intl. Plant Prop. Soc. 30:421-7.

Popovich, S. 1982. Performance of hybrid poplars in Quebec: ten years after planting. Laurentian Forest Research Centre. Publication LAU-X-44. 21 p.

Sutter, E. G. and P. B. Barker. 1985. In vitro propagation of mature Liquidambar styraciflua. Plant Cell Tissue Organ Culture 5:13-21.

Vann, S. R., G. W. Wyckoff, and J. W. Wyckoff. 1987. A tissue culture solution to a forestry problem - the propagation of a tetraploid European aspen. IPC Tech. Paper Series 264. 4 p.

Wilkinson, R. C. 1973. Inheritance and correlation of growth characters in hybrid poplar clones. Proceedings 20th Northeastern Forest Tree Improvement Conference, Durham, New Hampshire. p. 121-130.

Wyckoff, G. W., Einapshr, D. W., and Dinus, R. J. 1987. Growth and survival of hybrid poplar and Populus deltoides clones in East Central Wisconsin. Proceedings 5th North Central Tree Improvement Conference, Fargo, North Dakota. p. 187-196.

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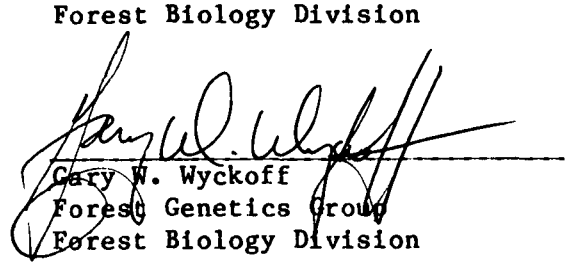

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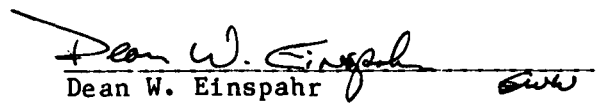

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APPENDIX

A code system was devised early in the aspen program to handle the numbering of individual parent trees and crosses. It was necessary to incorporate into the system the ability to identify the species of selected parent trees, the type of cross (parentage) when used for crosses, and the year the trees were selected or the cross was made. The following list alphabetically gives the symbols encountered in the selected tree and crossing system.

To illustrate, T-2-56 = the second *P. tremuloides* selected in 1956. XT-Ta-14-58 is the 14th cross made in 1958 and the cross involves a *P. tremuloides* female and a *P. tremula* male. XCa-G-18-70, S-1 = the first selected individual from the 18th cross in 1970 involving a *P. canescens* female and a *P. grandidentata* male.

A = *P. alba*

An = *P. angustifolia*

B = *P. alba* var. bolleana

Ca = *P. canescens*

D = *P. deltoides*

Da = *P. davidiana*

E = *P. euphratica*

G = *P. grandidentata*

Gla = *P. glandulosa*

H = hybrid

I = *P. ilicifolia*

M = *P. maximowiczii*

N = *P. nigra*

O = open pollinated

S = *P. sieboldii*

S-1, S-2, S-3, ... = selected individuals

T = *P. tremuloides*

Ta = *P. tremula*

Tc = *P. balsamifera*

Tr = *P. trichocarpa*

Ts = *P. tristis*

X = cross

GLOSSARY

Aspen. Refers to P. davidiana, grandidentata, sieboldii, tremula, and tremuloides. As used in this report, refers to species in the Populus section Leuce, which includes the aspens (subsection Trepidae Dode) and the true white poplars (subsection Albidae Dode) which includes P. alba and P. canescens. Hybrids of species within this section are also covered by this term. (See poplar.)

Auxins. A class of growth hormones causing cell enlargement.

Bh. Breast height (4.5 feet).

Bioassay. Determination of the relative effective strength of a substance by evaluating its effect on a test organism.

Bisexual. Having both functional male and female reproductive organs in the same flower, or in the case of Populus, a tree having both male and female flowers.

Canker. A necrotic area caused by fungi or bacteria. Hypoxylon cankers have a flat, sunken appearance that may or may not have callus ridges along the margin of the necrotic area.

Catkin. A scaly spike of usually unisexual flowers, as in Betula and Populus.

Chromosome. A microscopic, usually thread- or rodlike body carrying the units of inheritance (genes). The chromosomes are the primary constituents of the cell nucleus but are individually distinguishable only during nuclear division.

Chromosome number. The number or complement of chromosomes characteristic of a species. The number of sets must also be specified; thus, in Pinus the chromosome number may be expressed as "n equals 12" or as "2n equals 24," depending on whether sex cells or vegetative cells are observed.

Chromosome set. The chromosomes inherited as a unit from one parent. Most eggs or sperm carry only one set. A set usually includes one of each kind of chromosome characteristic of the species.

Clone. A group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Cross. As used in the Aspen Genetics Program the term applies to progeny produced by mating trees of the same species (intraspecific) or of different species (interspecific).

Dbh. Diameter of the tree stem at breast height (4.5 feet).

Diploid. Having two sets of chromosomes in the nucleus - indicated by "2n." One-half of the chromosomes are contributed by the female parent, one-half by the male parent. Many higher organisms are diploid except for their sex cells and associated tissue.

Fertilization. Union of a haploid male sex cell with a haploid female egg cell to form a diploid zygote which develops into the normal tree.

Gene. The smallest unit that can be shown to be consistently associated with the occurrence of a specific genetic effect. The genes are ultramicroscopic and act as if linearly arranged at fixed places (loci) on the chromosomes. Each gene interacts with other genes and the environment to produce within the cell certain physiological effects that control the development of one or more characters of an individual.

Genotype. An individual's hereditary constitution, expressed or hidden, underlying one or more characters; the gene classification of this constitution expressed in a formula. The genotype is determined chiefly from breeding behavior and ancestry.

Haploid. Having the reduced chromosome number (n), i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half of the number of sets occurring in diploid (2n) vegetative cells.

Heritability. A measure of the relative degree to which a character is influenced by heredity as compared to environment. The heritability (in the narrow sense) of a character in a population is the fraction of the total variation that is contributed by transmissible (additive) genetic differences, i.e., it is the ratio of genotypic variance to phenotypic variance. High heritability indicates that an individual's phenotype is indicative of its genotype and that differences in environment will cause little modification, i.e., that genetic control is high.

Heterosis. Hybrid vigor; the increased vigor of a hybrid as compared to the better parent. Heterosis is at a maximum in the F1 generation.

Heterozygosity. Presence in an organism of different members of the same allelic set, i.e., both the dominant and the recessive gene. For example, an Aa plant is heterozygous, whereas AA and aa plants are homozygous. A heterozygous individual characteristically does not breed true and is known as a hybrid with respect to the genes in question.

Homozygosity. Presence of identical alleles, either both dominant or both recessive, as for example AA or aa. A homozygous individual breeds true when mated with the same genotype for the character(s) in question.

Hybrid. As used in the Aspen Genetics Program the term applies to progeny produced as the result of mating trees of different species (interspecific).

Hybrid vigor. Same as heterosis.

Inbreeding. Intercrossing or selfing related organisms. This procedure, especially if carried out for a number of generations, exposes undesirable recessive characters and "fixes" desirable ones, i.e., renders them true-breeding.

Interspecific. Between species; e.g., interspecific hybridization is the production of offspring by cross-pollinating one species with another.

Intraspecific. Within a species; e.g., intraspecific hybridization is the production of offspring by cross-pollinating one individual of a species with pollen from another individual of the same species.

Mammatoxin. A term describing a poisonous substance produced by the fungus Hypoxylon mammatum.

Mutation. A sudden variation from the ancestral phenotype, due to gene or chromosome changes. If the cause can be demonstrated as a chromosome change, the mutation is preferably referred to by the specific phenomenon involved, e.g., a change in structure (aberration) or number (ploidy). Although mutations are infrequent, and usually recessive and harmful, they are the raw material of evolution and plant breeding.

Nucleus. The cell part made up chiefly of the chromosomes.

Ortet. The one plant from which members of a clone were originally derived.

Pathotoxin. A poisonous substance that is a product of metabolic activities.

Phenotype. (1) The demonstrable characteristic(s) of an organism; the product of the interaction of the genes of an organism with the environment. (2) Individual(s) described on the basis of demonstrable characteristics. Similar phenotypes do not necessarily breed alike.

Plantlet. A complete plant derived from a tissue culture system.

Ploidy. The chromosome situation with respect to number of sets, e.g., two sets (diploid), or variation from full sets (aneuploid).

Pollination. When pollen reaches the receptive catkin.

Polyploid. Having three or more times the haploid number of chromosome sets in its cells. A cell or organism having three sets (3n) is called triploid; four sets (4n) tetraploid.

Poplars. Refers to trees in the genus Populus. As used in this report, refers to species outside the section Leuce (see aspen).

Popple. A colloquialism which refers to native aspen, P. tremuloides and P. grandidentata.

Progeny test. Evaluation of the breeding value of parents by suitable comparisons among their offspring.

Putative. Suspected.

Ramet. An individual member of a clone.

Reciprocal cross. The repetition of a cross where the sexual function of the parents is reversed, i.e., female A x male B is the reciprocal of female B x male A.

Selection. Artificial selection is the propagation by man of organisms possessing desired characteristics. The aim generally is to improve the population or gain knowledge of its hereditary potentials. Natural selection is part of the evolutionary process resulting in the survival of the "fittest," i.e., of the best adapted individuals.

Sibs (siblings). Offspring, irrespective of sex, from the same parents but from separate fertilizations. Full sibs have both parents in common, half-sibs, only one in common.

Sprout. Vegetative shoot arising from the stump or roots. Root sprouts may also be designated as suckers.

Suckers. Vegetative shoots arising from subterranean roots or stems.

Tetraploid. See polyploid.

Tissue culture. A general term for organs, callus, or cells growing in vitro on an artificial medium. Cultures can be started from a variety of plant parts which have cells capable of dividing.

Triploid. See polyploid.

Vegetative propagation. Propagation of a plant by asexual parts, as in tissue culture, budding, dividing, grafting, rooting, and air layering. Hereditary characteristics of the resulting clone (ramets) are identical with those of the original plant (ortet).